Plant Physiol. (1993) 101: 773–779

An Osmotic Stress Protein of Cyanobacteria Is Immunologically Related to Plant Dehydrins[1]

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Dehydrins are a family of desiccation proteins that were identified originally in plants (T.J. Close, A.A. Kortt, P.M. Chandler [1989] Plant Mol Biol 13: 95–108; G. Galau, T.J. Close [1992] Plant Physiol 98: 1523–1525). Dehydrins are characterized by the consensus amino acid sequence domain EKKGIMDKIKEKLPG found at or near the carboxy terminus; the core of this domain (KIKEKLPG) may be repeated from one to many times within the complete polypeptide. Dehydrins generally accumulate in plants in response to dehydration stress, regardless of whether the stimulus is evaporation, chilling, or a decrease in external osmotic potential. Polyclonal antibodies highly specific to the consensus carboxy terminus of plant dehydrins were used to search for dehydrins in cyanobacteria, many of which are known to survive desiccation. A 40-kD osmotic-stress-induced protein was identified in *Anabaena* sp. strain PCC 7120. The 40-kD protein was usually not detected in logarithmic cultures and was induced by shifting the growth medium to higher solute concentrations. Several solutes have inductive effects, including sucrose, sorbitol, and polyethylene glycol (PEG). Measurements of osmotic potential suggest that a shift of −0.5 MPa (sucrose and PEG) or −1.2 MPa (sorbitol) is sufficient to induce synthesis of the 40-kD protein. Glycerol, which is highly permeable, was not an inducer at −1.2 MPa (0.5 M), nor was the plant hormone abscisic acid. Induction appears to be evoked by a shift in osmotic potential approximately equal in absolute magnitude to the expected turgor pressure of bacterial cells in logarithmic phase growth. A dehydrin-like polypeptide was also identified among osmotically induced proteins from two other filamentous, heterocyst-forming cyanobacteria. A 40-kD protein was observed in *Calothrix* sp. strain PCC 7601, and in *Nostoc* sp. strain Mac-R2, an osmotic-induced doublet at 39 and 40 kD was observed. From these data, it appears that cyanobacteria produce a dehydrin-like protein under osmotic stress.

The responses of living organisms to abiotic stress show remarkable similarities over a wide evolutionary distance. This is particularly true of biochemical adaptations operating at the cellular level, as exemplified by the well-known heat-shock response (Vierling, 1991; Zeilstra-Ryalls et al., 1991). Because drought or water stress is likely to have been as pervasive as heat shock during the evolution of life on this planet, we hypothesized that successful adaptive strategies for water-limited environments would be as conserved as the heat-shock response. Such conservation would be detectable in the primary structure of proteins functioning in water-stress adaptation. We report here on the presence of osmoregulated proteins in cyanobacteria that are immunologically related to a family of higher plant desiccation proteins known as dehydrins.

Dehydrins are a family of proteins characterized by the amino acid consensus sequence KIKEKLPG, which is found one to many times within the complete dehydrin protein sequence (Galau and Close, 1992). Dehydrins generally accumulate in plants in response to cellular dehydration, regardless of whether dehydration is induced during seed development or by evaporation, cold temperature, or osmotic stress (Skriver and Mundy, 1990; Gulick and Dvorak, 1992; Houde et al., 1992). A relationship among tolerance to drought, salt, and freezing in plants has been considered in the literature since early in this century (reviewed by Siminovitch and Cloutier, 1983). Each of these stresses results in a lower chemical potential of water external to the cell and thus drives the net movement of water outward across the plasma membrane, literally causing dehydration. The result of rapid water removal is a loss of turgor, followed by a decrease in cell volume accompanied by concentration of internal solutes. Contraction of the plasma membrane may also occur. Dehydrins typically accumulate during the normal process of embryo desiccation, and their appearance may be induced by the plant stress hormone ABA. Dehydrins have been referred to by other names in the plant literature, including LEA group 2 proteins and RAB proteins (reviewed by Dure et al., 1989; Skriver and Mundy, 1990; Galau and Close, 1992). Immunological studies have established the presence of dehydrins in mature seeds of a wide range of plants, including both angiosperms and gymnosperms (T.J. Close, unpublished data).

The rationale for comparing the water-stress response in plants and cyanobacteria is 2-fold. In addition to being the probable progenitors of higher plant chloroplasts (Gray and Doolittle, 1982), many cyanobacteria exhibit remarkable tolerance to desiccation. This phenomenon has been particularly well studied at structural, physiological, and biochemical levels in *Nostoc commune* (Potts, 1986; Scherer and Potts, 1989). The ability to withstand desiccation appears to be somewhat common among freshwater filamentous cyanobacteria including most commonly studied *Anabaena, Nostoc,*

[1] Supported by the Southwest Consortium on Plant Genetics and Water Resources grant No. 88-34186-3340 to T.J.C. and P.J.M.

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Abbreviations: Ig, immunoglobulin; LEA proteins, late embryogenesis-abundant proteins.

and *Calothrix* strains (P.J. Lammers, unpublished data). Changes in protein profiles have been demonstrated during osmotic stress of salt-tolerant and salt-sensitive strains of *Anabaena* (Apte and Bhagwat, 1989). Comparison of desiccated versus liquid-grown cells of *N. commune* has revealed differences in the concentrations of several proteins. One of these, a very abundant 39-kD acidic protein, has been purified, and a partial N-terminal amino acid sequence has been reported (Scherer and Potts, 1989).

Some similarities in the response of plants and bacteria to water stress are well established. In both plants and bacteria, it is widely accepted that intracellular solute accumulation serves as a means of maintaining turgor as the chemical potential of water in the environment decreases. In the simplest case, turgor pressure is the difference between the chemical potential of water inside and outside the cell. Extensive similarities exist in the types of solutes utilized by plants and bacteria; Pro, Gly betaine, various simple sugars, and, to some extent, potassium ion, appear to be compatible with cellular metabolism (Csonka and Hanson, 1991). Several compatible solutes are known in the cyanobacteria, including trehalose, Gly-betaine, glucosyl-glycerol, Pro, and Suc. Suc is a common solute accumulated in freshwater strains, and most *Anabaena* and *Nostoc* strains accumulate Suc during osmotic stress (Reed et al., 1984).

Anabaena 7120 is the most commonly studied filamentous cyanobacterium because of its ability to fix nitrogen in differentiated heterocysts and the facility with which genetic manipulations can be accomplished in this strain (Elhai, 1987; Cai and Wolk, 1990; Haselkorn, 1991). *Anabaena* 7120 cells remain viable for at least 1 year on dehydrated agar plates maintained in the dark at room temperature. Microscopic examination of cells on dehydrated agar chips reveals healthy green pigmentation and essentially normal morphology. Vigorous growth is apparent soon after rehydration in fresh liquid medium. These results suggest that desiccation tolerance can be effectively studied in *Anabaena* 7120. Using antibodies against a dehydrin consensus peptide, we examined *Anabaena* 7120 and two additional filamentous cyanobacteria for the presence of proteins cross-reactive with plant dehydrins.

MATERIALS AND METHODS

Growth of Bacterial Cultures

All strains were maintained and grown in BG-11 medium (Rippka, 1988). For most experiments, $NaNO_3$ was replaced by 2.5 mM $(NH_4)_2SO_4$. Cultures were grown under continuous light at 28 to 30°C. Liquid cultures were bubbled with a 1% (v/v) CO_2/air mixture and buffered with 5 mM Mops. *Nostoc* sp. strain Mac-R2 is a fix^+ revertant of *Nostoc* sp. strain PCC 7911 obtained from J.C. Meeks (University of California, Davis). *Calothrix* sp. strain PCC 7601 and *Anabaena* sp. strain PCC 7120 were obtained from the collection of R. Haselkorn (University of Chicago).

Osmotic Stress of Cyanobacterial Cultures and Preparation of Protein Extracts

Cultures were grown to late log phase, harvested at a density between 12 and 15 μg/mL Chl *a* by sterile centrifugation, and resuspended in one-fifth the harvested volume of fresh BG-11 medium with or without various osmotica. PEG-8000 was obtained from Sigma Chemical Co. The resuspended cultures were further incubated for the indicated time under the same light, temperature, and stirring regimes used throughout, but without 1% CO_2 bubbling. Treated and control culture samples (60–75 μg/mL Chl *a*) were harvested by centrifugation, resuspended in one-fifth volume of standard Laemmli loading buffer (Harlow and Lane, 1988), quick frozen in liquid nitrogen, and stored frozen at −70°C (25-fold overall concentration factor).

Proteins in 35 μL of these lysates were heated to 100°C and electrophoresed on mini-protein gels (SDS-PAGE with 12% polyacrylamide) using Mini-Protean II Electrophoresis Cells (Bio-Rad). Protein bands in duplicate gels were visualized using Coomassie blue R-250 dye or by silver staining (Harlow and Lane, 1988). Prestained protein standards (Bio-Rad) included the following proteins (with their apparent modified molecular mass): rabbit muscle phosphorylase B (110 kD), BSA (84 kD), hen egg white ovalbumin (47 kD), bovine carbonic anhydrase (33 kD), soybean trypsin inhibitor (24 kD), and hen egg white lysozyme (16 kD). Extracts from corn (*Zea mays* L.) or barley (*Hordeum vulgare* L.) containing plant dehydrins were included as positive controls. For these controls, 8 μg of total protein was used, as determined by a dye-binding assay using bovine gamma globulin (Bio-Rad) as a standard (Harlow and Lane, 1988).

Anti-Dehydrin Antibodies

Full details of the method used to produce highly specific polyclonal anti-dehydrin antibodies will be published elsewhere (T.J. Close, unpublished data). In brief, a synthetic peptide including the amino acid sequence KIKEKLPG was coupled to BSA and the conjugate was used to immunize rabbits. Anti-peptide antibodies were affinity purified from crude serum using a gel matrix to which the synthetic peptide had been coupled. The purity of anti-peptide IgG was assessed by comparing it with crude preimmune and immune sera using SDS-PAGE, ELISA, and immunoblotting. Affinity purification resulted in a preparation that was approximately 90% IgG, contained less than 1% of the original nonspecific antibodies, and recognized all cereal dehydrins that were recognized by the crude serum. IgG concentrations stated in the figure legends refer to the total protein present in the IgG preparations, which will overestimate the IgG by about 10%.

Immunoblots

Proteins were transferred electrophoretically to nitrocellulose in Mini Trans-Blot Cells (Bio-Rad). The filters were blocked with 3% (w/v) gelatin in Tris-buffered saline, incubated with rabbit anti-peptide antibodies, and then incubated with goat anti-rabbit IgG alkaline phosphatase conjugate (Fisher) essentially as described previously (Harlow and Lane, 1988). Secondary antibody was detected using 4-nitrobluetetrazolium chloride and 5-bromo-4-chloro-3-indolyl-phosphate (Harlow and Lane, 1988).

Osmotic Potential Measurements

The osmotic potentials of growth media containing various solutes were measured independently using a Wescor 5100C vapor pressure osmometer and JRD Merrill thermocouple psychrometers linked to a Campbell CR-7 Datalogger. The osmometer and psychrometers were calibrated with NaCl and Suc solutions.

ABA Assays

ABA levels were determined on sonicated extracts of *Anabaena* cells using a monoclonal antibody assay as described by Walker-Simmons (1987).

RESULTS

The Strategy for Inducing Dehydrins in Liquid Cultures of Cyanobacteria

Because dehydrins are generally associated with stress in plants, our initial hypothesis was that they would also be associated with stress in bacteria. For this reason, we sought conditions that would cause the withdrawal of water from bacterial cells without severely limiting their ability to transcribe mRNA and translate proteins. With liquid cultures of bacteria, this can be achieved quite easily by simply decreasing the osmotic potential of the growth medium. Because the loss of turgor seems to be the stimulus of many plant dehydration stress responses, including ABA biosynthesis (Pierce and Raschke, 1980), the range of osmotic potential adjustment was designed to gradually approach, and then exceed, the turgor pressure in growing *Anabaena* cells. In log phase cultures of some Gram-negative bacteria, turgor is known to be in the range of about 0.5 MPa (Csonka and Hanson, 1991). Suc is not metabolized by most cyanobacteria and cannot be transported rapidly to balance the internal and external osmotic pressures (Walsby, 1988). Taking advantage of this property, Suc was utilized as an uncharged osmoticum to manipulate the osmotic potential of cyanobacterial growth media. We then screened for cyanobacterial dehydrin-like proteins (cyano-dehydrins) using polyclonal affinity-purified anti-dehydrin-peptide antibodies (see "Materials and Methods").

Osmotic Stress-Induced Changes in Protein Profiles

The consequences of osmotic stress treatments on *Anabaena* 7120 were monitored first by observing protein profiles from cultures subjected to increasing concentrations of Suc for 48 h. Figure 1 shows that one major Suc-induced protein is observed with an M_r near 35,000. This protein is quite prominent in cultures treated with 250 mM Suc, very intense in cultures treated with 500 mM Suc, but not apparent in cultures treated with 1.0 or 1.5 M Suc. The shift to 1 M or higher Suc seemed to cause a general metabolic disruption and partial cell lysis; therefore, only the lower Suc levels are meaningful in terms of adaptive changes in proteins.

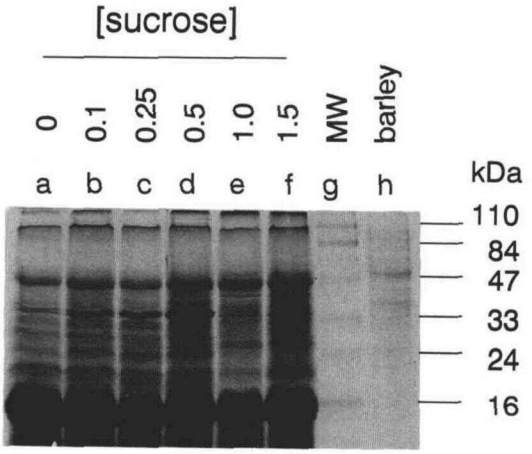

Figure 1. Protein profiles from osmotically stressed *Anabaena* 7120 cultures. SDS-PAGE and Coomassie blue staining (see "Materials and Methods"). Lanes a through f contained extracts of cultures shifted to increasing concentrations of Suc, shown in molarity. Lane g contained molecular mass markers, with approximate sizes indicated to the right. Lane h contained proteins from scutella of dehydration-stressed Himalaya barley seedlings, which are a source of barley dehydrins.

Immunological Detection of *Anabaena* 7120 Dehydrin-Like Protein

Proteins from each of the above treatments were electroblotted to nitrocellulose and probed immunologically. Preimmune serum reacted with many proteins in the bacterial extracts, including constitutive and stress-induced bands (Fig. 2A). All of these reactions were assumed to be nonspecific protein-Ig interactions. The positive control barley protein extracts did not react significantly with the preimmune serum. As shown in Figure 2B, crude anti-dehydrin peptide serum identified barley dehydrins, a more complete description of which will be discussed elsewhere (T.J. Close, unpublished data). In *Anabaena* extracts, many of the same bands were recognized by both the preimmune and the crude immune sera, and there were many additional bands that generally had no correlation with Suc concentrations (Fig. 2, compare A and B). However, within this heavy background of bands, a Suc-induced band at 40 kD was clearly detected by immune serum, but not by preimmune serum.

In marked contrast, affinity-purified anti-dehydrin IgG reacted strongly with only one protein band of approximately 40 kD (Fig. 2C). The intensity of the 40-kD band identified by the affinity-purified IgG fraction increased with the osmotic strength of the growth medium up to 500 mM Suc. The induction by increased osmoticum is quite reproducible, because we have observed osmotic induction of the 40-kD protein in eight independent experiments with *Anabaena* 7120. However, detection of the 40-kD dehydrin-like protein in zero Suc- or 100 mM Suc-treated cultures has been somewhat variable for reasons that we do not yet understand (compare Figs. 2C and 3). We concluded from the data in Figure 2 that *Anabaena* 7120 contains an osmotic-stress-protein that is immunologically related to the plant dehy-

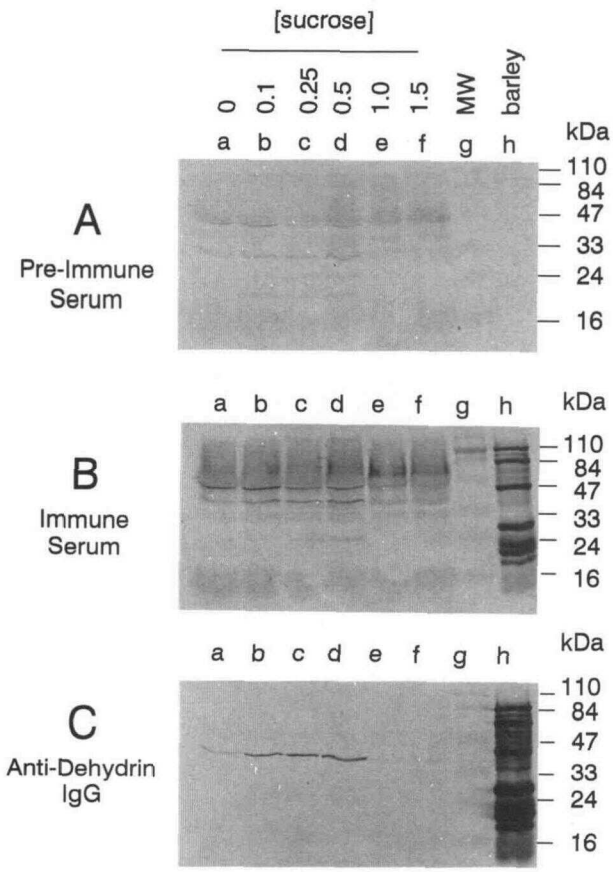

Figure 2. Western blot evidence of *Anabaena* 7120 dehydrins. SDS-PAGE with the same samples used for Figure 1, testing the effect of increased Suc concentrations in the medium. Proteins were transferred to nitrocellulose and examined with various immunological materials prepared from a single rabbit. A, Preimmune serum diluted 1/50; B, crude immune serum diluted 1/50; C, affinity-purified IgG diluted to 7 μg/mL. Incubations in primary antibody were for 16 h at 25°C.

drins. We refer to the 40-kD band as cyano-dehydrin to indicate this similarity.

Timing and Specificity of Cyano-Dehydrin Induction in Anabaena 7120

The time course of induction of cyano-dehydrin in *Anabaena* 7120 by Suc and an additional osmoticum, sorbitol, is shown in Figure 3. Both treatments induced the 40-kD cyano-dehydrin after 6 h, but with sorbitol, twice the concentration was necessary for an inductive effect. The osmotic potential of the starting BG-11 media was −0.03 MPa, the 0.25 M Suc medium was −0.53 MPa, and the inducing sorbitol medium (0.5 M) was −1.28 MPa. Sorbitol induction was also more transient than Suc induction, as indicated by the disappearance of the cyano-dehydrin band 24 h after sorbitol treatment.

To establish more firmly the link between osmotic stress and cyano-dehydrin accumulation, we tested a known non-permeating solute, PEG, and one rapidly permeating solute, glycerol. We predicted that because glycerol equilibrates across biological membranes by passive diffusion, a very transient drop in turgor pressure would result that may not lead to prolonged accumulation of cyano-dehydrin. Conversely, the larger size of the PEG molecules effectively prevents transmembrane equilibration and would induce osmotic stress responses. As shown in Figure 4, the 40-kD cyano-dehydrin from *Anabaena* 7120 was enhanced in the 20% PEG-treated sample. The measured solute potential of this sample was −0.53 MPa, which was very close to the solute potential of the 0.25 M Suc culture. As we predicted, glycerol had no effect up to 0.5 M (−1.2 MPa) in a 5-h stress experiment. We cannot exclude a rapid transient induction of the 40-kD protein in response to glycerol. It should also be noted that in this experiment, 100 mM Suc (−0.20 MPa) and 10% PEG (−0.10 MPa) were not effective inducing conditions. These experiments demonstrate that the induction is related to a threshold osmoticum and not to an elevated concentration of a carbon or energy source.

Role of ABA, pH, and Temperature in Induction

Plant dehydrin genes have been shown in many cases to be regulated by the hormone ABA, and ABA has been

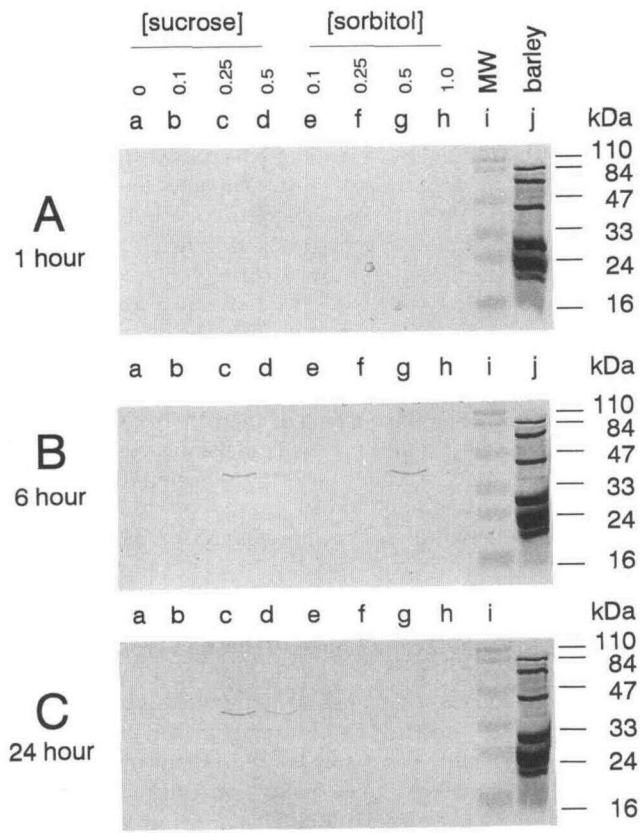

Figure 3. Osmotic-stress induction of cyano-dehydrin in *Anabaena* 7120. *Anabaena* samples and immunoblots were prepared essentially as in Figure 2, but with additional cultures shifted to 0 through 0.5 M Suc (lanes a-d) or 0 through 1.0 M sorbitol (lanes a, e-h). The control lanes are i and j, as in Figure 2. Times after culture shift are indicated as 1, 6, and 24 h in panels A to C, respectively.

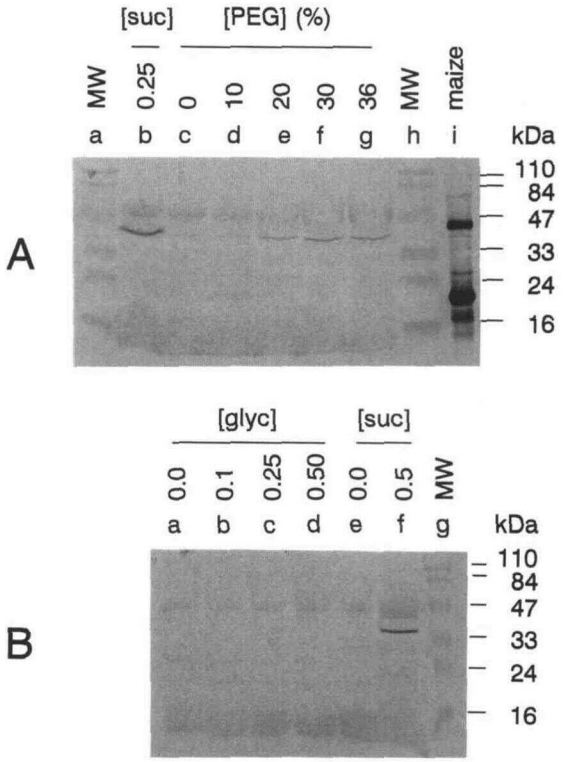

Figure 4. Nonpermeable solute induction of the 40-kD cyano-dehydrin. *Anabaena* samples and immunoblots were prepared essentially as in Figures 2 and 3, but alternative solutes were used. A, Results of 0 to 36% PEG-8000 (lanes c-g); B, 0 to 0.5 M glycerol (lanes a-d). Control lanes include prestained molecular mass markers (A, lanes a and h; B, lane g), proteins from embryos of imbibed maize B73 kernels, which is a source of maize dehydrins (A, lane i), and 0.0 and 0.25 M Suc-induced *Anabaena* proteins (B, lanes e and f), as shown in Figure 2B.

reported to be present in various cyanobacteria (Hirsch et al., 1989). Measurement of the ABA levels in *Anabaena* 7120 revealed very low levels of ABA that did not exceed 0.2 μM and did not change with increasing Suc (data not shown). Furthermore, addition of up to 1 mM ABA to unstressed cultures did not induce the 40-kD cyano-dehydrin (data not shown). It seems unlikely, therefore, that ABA signals water stress in *Anabaena* 7120, and ABA apparently does not regulate cyano-dehydrin expression in *Anabaena* 7120.

We also sought to determine if the induction of the *Anabaena* 7120 dehydrin-like protein is limited to osmotic stress, or if other abiotic stresses have an inductive effect. High and low pH and temperature shifts were tested in 4-h stress treatments. The pH shifts (4.0, 5.0, 9.0, or 10.0) and 10°C temperature shifts (20°C or 40°C) failed to induce cyano-dehydrin to levels observed after osmotic stress. However, both the temperature shifts and low pH resulted in a small degree of induction that warrants additional study (data not shown).

Cyano-Dehydrin Is Present and Osmotically Induced in Other Filamentous Cyanobacteria

Based on the above studies with *Anabaena*, we reasoned that if dehydrins are in fact a broadly distributed class of osmotic-stress proteins, then the antibodies used for the *Anabaena* studies might also be useful for demonstrating the existence of dehydrins in other cyanobacteria and for determining the extent of the conservation of cyano-dehydrin induction by water stress. The results of such a study are shown in Figure 5. Affinity-purified anti-dehydrin peptide IgG identifies osmotically induced proteins from two additional genera of filamentous cyanobacteria. *Calothrix* sp. strain PCC 7601 shows an osmotically induced 40-kD band, and a 39- to 40-kD doublet was observed in cultures of *Nostoc* sp. strain PCC 7911 (Mac-R2). In both organisms, the amount of cyano-dehydrin increased in response to osmotic stress, as previously shown for *Anabaena* 7120. The basal level of the protein in unstressed cultures of *Nostoc* Mac-R2 was higher than that observed in the experiment with *Calothrix* 7601 and similar to levels seen in some experiments with *Anabaena* 7120. In essence, there appear to be cyano-dehydrins in *Calothrix* and *Nostoc*, as well as *Anabaena*, and in each case, there is at least some induction following osmotic shift. We feel that these results warrant a further search for dehydrin-like proteins in other nonplant species.

DISCUSSION

Dehydrins are a family of proteins originally discovered in plants and characterized by the conserved amino acid sequence motif EKKGIMDKIKEKLPG (Galau and Close, 1992). The occurrence of dehydrins in plants has in many cases been correlated with drought, salt, and cold adaptation. However, a cause and effect relationship between plant dehydrin accumulation and stress adaptation has never been established. This situation is due in large part to the difficulty of performing directed mutational analyses in plants, which precludes the elegant genetic studies that are routine in microorganisms. Our search for dehydrins in microorganisms was motivated in part by the great potential for the use of microorganisms in functional analyses of dehydrins and other osmotic stress proteins.

In this article, we have presented evidence for osmotic-stress-induced proteins of cyanobacteria that are immunologically related to plant dehydrins. Antibodies specific to the dehydrin-conserved sequence recognized proteins from three genera of filamentous cyanobacteria; *Anabaena*, *Calothrix*, and *Nostoc*. The M_r of the cyano-dehydrins were remarkably similar. *Anabaena* 7120 and *Calothrix* 7601 both exhibit a 40-kD band, and a doublet of 39 and 40 kD was observed in *Nostoc* Mac-R2. The cyano-dehydrin protein is clearly distinct from a major Suc-induced protein in *Anabaena* 7120 that has an M_r of approximately 35,000. This latter protein may be a relative of the major desiccation-induced protein with a slightly greater M_r that was purified by Scherer and Potts (1989) from *N. commune*. Furthermore, J. Curry and M.K. Walker-Simmons (personal communication) have both nucleic acid hybridization and immunological evidence that a Group 3 LEA counterpart may exist in *Anabaena*. As described above, Group 3 LEA proteins are distinct from dehydrins (Group 2 LEAs). These results are consistent with the hypothesis that some adaptations to osmotic stress have been conserved at the protein level over large evolutionary distances. Our results also suggest that it may be possible to use

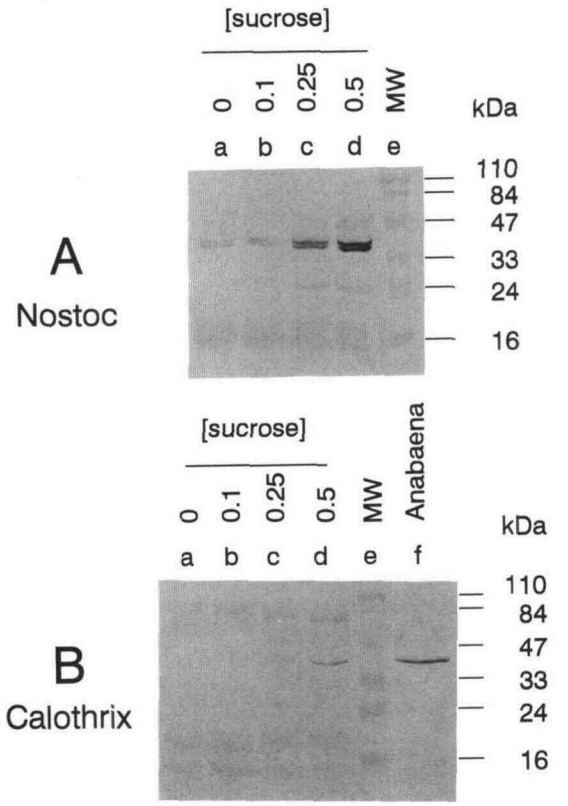

Figure 5. Induction of cyano-dehydrins in other cyanobacteria. Suc induction (0–0.5 M) of cultures of *Nostoc* (A, lanes a-d) and *Calothrix* (B, lanes a-d) was essentially the same as in Figure 3. Samples and immunoblots were prepared as in Figures 2 through 4. Control lanes in both panels included prestained molecular mass markers (A and B, lane e) and 0.25 M Suc-induced *Anabaena* proteins (B, lane f), as shown in Figure 2B.

cyanobacteria, and possibly other microorganisms, to study the fundamental properties of dehydrins.

In the studies presented in this article, we used only nonionic solutes for induction of the dehydrin-like protein. We chose not to use ionic solutes such as NaCl because small ions affect not only the osmotic potential, but also act as electrolytes and may have additional toxicities in the intracellular environment. Our aim was to investigate only the influence of osmotic potential. As a result, Suc, sorbitol, and PEG all induced the 40-kD cyano-dehydrin, and, as expected, we did not observe an induction of cyano-dehydrin in response to the highly permeable glycerol molecule. It is most interesting to note that for two different solutes, Suc and PEG, the magnitude of the change in solute potential necessary to induce the accumulation of cyano-dehydrin (−0.5 MPa) is about equal to, or may slightly exceed, the turgor pressure values (0.5 MPa) observed in some log-phase cultures of Gram-negative bacterial cells (Csonka and Hanson, 1991). Furthermore, a shift of only 0.2 MPa appears to be insufficient for induction.

We do not know the exact turgor pressure values for *Anabaena* cells growing under our conditions. Nonetheless, we interpret our threshold osmotic potential data to indicate that the loss of turgor, or a substantial reduction in turgor, is adequate to induce cyano-dehydrins; extensive dehydration of the cytoplasm is apparently not required. One rationale for the more transient behavior of sorbitol is that sorbitol may be more permeating than Suc, resulting in a gradual equilibration of intracellular and extracellular sorbitol in *Anabaena* 7120. This perhaps produces a more transient reduction of turgor than that caused by Suc.

In addition to the structural relationship defined by cross-reaction with affinity-purified antibodies, a physiological relationship between plant dehydrins and cyano-dehydrins is evident in the regulation of cyano-dehydrin accumulation by osmotic stress. Together, these similarities lead us to the simple interpretation that the fundamental properties of plant dehydrins are probably also operating within desiccation-tolerant cyanobacterial cells during osmotic adaptation. Experiments to determine if dehydrins are present in desiccation-sensitive cyanobacteria are presently underway. Based on this line of reasoning, we suspect that homologs of other plant water-stress proteins may also be found in cyanobacteria if appropriate means are used to search for them. Judging from our apparent success with affinity-purified polyclonal antibodies raised against a conserved peptide of plant dehydrins, we suggest that the immunological approach taken in the present study may have general merit in addressing this question.

We are now purifying cyano-dehydrin protein from *Anabaena* 7120 for direct sequencing, and using the antibodies to screen expression libraries to identify the gene encoding the 40-kD protein. Sequence analysis will reveal the extent of primary structure similarity between what we have referred to here as cyano-dehydrin and known plant dehydrins. Once the gene endoding the *Anabaena* 40-kD protein has been cloned, insertional mutagenesis in *Anabaena* 7120 may afford the first phenotypic analysis of dehydrin mutants in any organism.

ACKNOWLEDGMENTS

The authors wish to thank Ray Fenton and Casilda Provencio for the excellent technical assistance and gratefully acknowledge the help of Michele Gottlieb in the use of the thermocouple psychrometers and the vapor pressure osmometer.

Received August 27, 1992; accepted November 14, 1992.

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